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Citation

Moriguchi, Hisashi, Yue Zhang, Makoto Mihara, and Chifumi Sato. 2012. A therapeutic method for the direct reprogramming of human liver cancer cells with only chemicals. *Scientific Reports* 2: 280.

Published Version

doi:10.1038/srep00280

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A therapeutic method for the direct reprogramming of human liver cancer cells with only chemicals

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SUBJECT AREAS:
TUMOUR SUPPRESSORS
DIFFERENTIATION
ONCOGENESIS
CANCER MODELS

Received
14 September 2011

Accepted
23 December 2011

Published
21 February 2012

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Various methods for the direct reprogramming of human somatic cells have been developed. However, a therapeutic method to reprogram and eliminate human solid tumor cells has not been developed. Here we show a novel therapeutic method to reprogram and eliminate human solid tumor cells with chemicals. This therapeutic method may be applicable to various human solid tumor cells that express aldo-keto reductase family 1 member B10 (AKR1B10) and retinoid X receptors (RXRs).

Various methods for the direct reprogramming of human somatic cells have been developed¹. However, therapeutic methods to reprogram and destroy human solid tumor cells have not been developed.

The acyclic retinoid (ACR) was found to reduce the rate of recurrence after curative hepatocellular carcinoma (HCC) therapy in a small randomized controlled trial ($n=89$)², and similar results were observed in a recent larger ($n=401$) randomized controlled trial³. The hazard ratio (ACR versus placebo) for 2 years or more after curative therapy was 0.27 (95% CI: 0.07–0.96)³. However, ACR failed to suppress recurrence within 1–2 years following curative therapy³. The hazard ratio (ACR versus placebo) for within 1 year of curative therapy was 0.72 (95% CI: 0.45–1.17)³. It has been postulated that aldo-keto reductase family 1 member B10 (AKR1B10)⁴ inhibits retinoic acid (RA)-induced cellular differentiation⁵; however, this hypothesis has not been proven.

Results

AKR1B10 expression in liver cancer cells from patients with HCC who received a liver transplantation. Following the methods previously described by our group and others⁴ (*cf. Methods*), we investigated the expression of AKR1B10 in liver cancer cells from 10 patients with HCC who received a liver transplantation⁶. AKR1B10 expression was observed in 6 of the patients with HCC (Figure 1a, 1b and Supplementary Figure 1a).

***In vitro* anticancer drug sensitivity testing in the 6 patients with HCC who expressed AKR1B10.** *In vitro* anticancer drug sensitivity testing was performed for the 6 patients with HCC who expressed AKR1B10 (*cf. Methods*). At 6 days, a significant difference was observed in the viability of AKR1B10-positive liver cancer cells treated with ACR alone and those treated with ACR plus zopolrestat, which is an AKR1B10 inhibitor⁷ (Figure 2A; $P<0.001$, Mann-Whitney *U* test). A significant difference was also observed at 6 days in the viability of AKR1B10-positive liver cancer cells treated with zopolrestat alone and those treated with ACR plus zopolrestat (Figure 2A; $P<0.001$, Mann-Whitney *U* test). Moreover, the mean alpha-fetoprotein (AFP) value at 3 days in samples treated with ACR alone was $45.3 \text{ ng} \pm 5.2 \text{ ng/L} \times 10^4 \text{ cells/24 h}$, while samples treated with ACR plus zopolrestat showed an improved value of $3.6 \text{ ng} \pm 2.2 \text{ ng/L} \times 10^4 \text{ cells/24 h}$ ($P<0.001$, Mann-Whitney *U* test). The mean albumin value at 3 days in samples treated with ACR alone was $19.3 \text{ ng} \pm 7.3 \text{ ng/L} \times 10^4 \text{ cells/24 h}$, while samples treated with ACR plus zopolrestat showed a significantly improved value of $93.3 \text{ ng} \pm 3.0 \text{ ng/L} \times 10^4 \text{ cells/24 h}$ ($P=0.02$, Mann-Whitney *U* test).

***In vitro* anticancer drug sensitivity testing in the 4 patients with HCC who did not express AKR1B10.** *In vitro* anticancer drug sensitivity testing was also performed in the 4 patients with HCC who did not express AKR1B10 (*cf. Methods*). At 6 days, a significant difference was observed in the viability of AKR1B10-negative liver cancer cells treated with ACR alone and those treated with ACR plus zopolrestat (Figure 2B; $P=0.02$, Mann-Whitney *U*

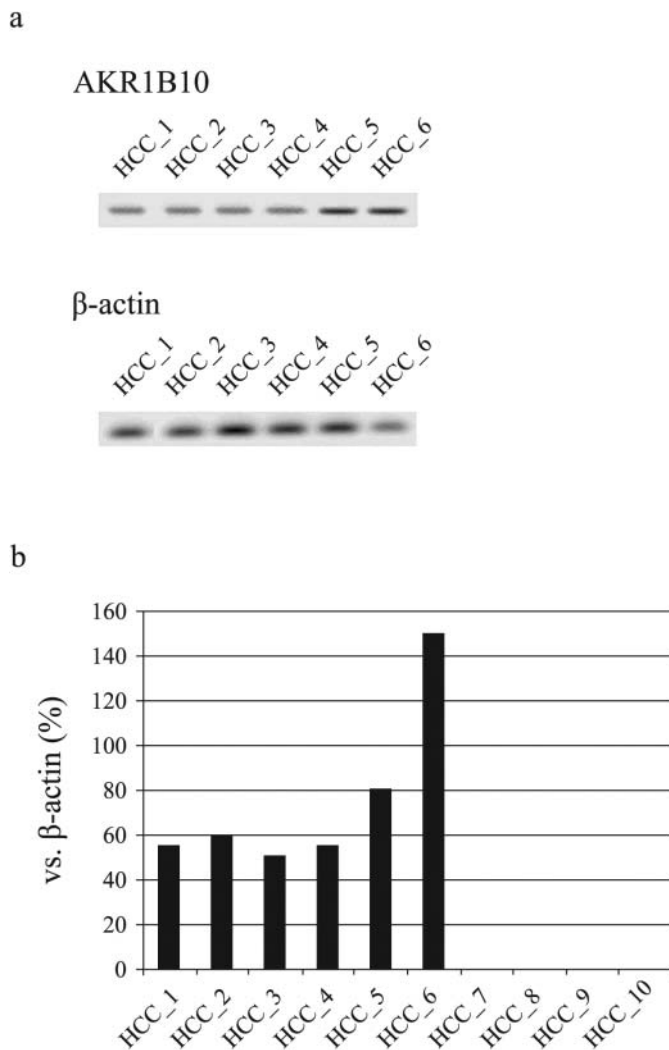


Figure 1 | (a) The expression of AKR1B10 by Western blot analysis in liver cancer cells from patients with HCC (n=6) who received a liver transplantation. Samples from the other patients (n=4) were AKR1B10-negative. β -actin is shown as a control. (b) Quantitative real-time PCR data of AKR1B10 in liver cancer cells from patients with HCC who received a liver transplantation (n=10).

test). A significant difference was also observed at 6 days in the viability of AKR1B10-negative liver cancer cells treated with zopolrestat and those treated with ACR plus zopolrestat (Figure 2B; $P < 0.001$, Mann-Whitney U test). Moreover, the mean AFP value at 3 days in samples treated with ACR alone was $50.3 \text{ ng} \pm 6.5 \text{ ng/L} \times 10^4 \text{ cells/24 h}$, while the mean value in samples treated with ACR plus zopolrestat was improved at $7.6 \text{ ng} \pm 3.5 \text{ ng/L} \times 10^4 \text{ cells/24 h}$ ($P = 0.01$, Mann-Whitney U test). The mean albumin value at 3 days in samples treated with ACR alone was $17.1 \text{ ng} \pm 5.2 \text{ ng/L} \times 10^4 \text{ cells/24 h}$, while the mean value in samples treated with ACR plus zopolrestat was significantly improved at $85.3 \text{ ng} \pm 3.9 \text{ ng/L} \times 10^4 \text{ cells/24 h}$ ($P = 0.02$, Mann-Whitney U test).

Oxygen consumption in liver cancer cells. Aerobic respiration in AKR1B10-positive liver cancer cells was assessed 3 days after the administration of ACR alone, zopolrestat alone or ACR plus zopolrestat. Oxygen consumption was significantly greater in the AKR1B10-positive liver cancer cells treated with ACR plus zopolrestat than in the cells treated with either ACR alone or zopolrestat alone (Figure 3A; $P < 0.001$, Mann-Whitney U test). Aerobic respiration in the AKR1B10-negative liver cancer cells

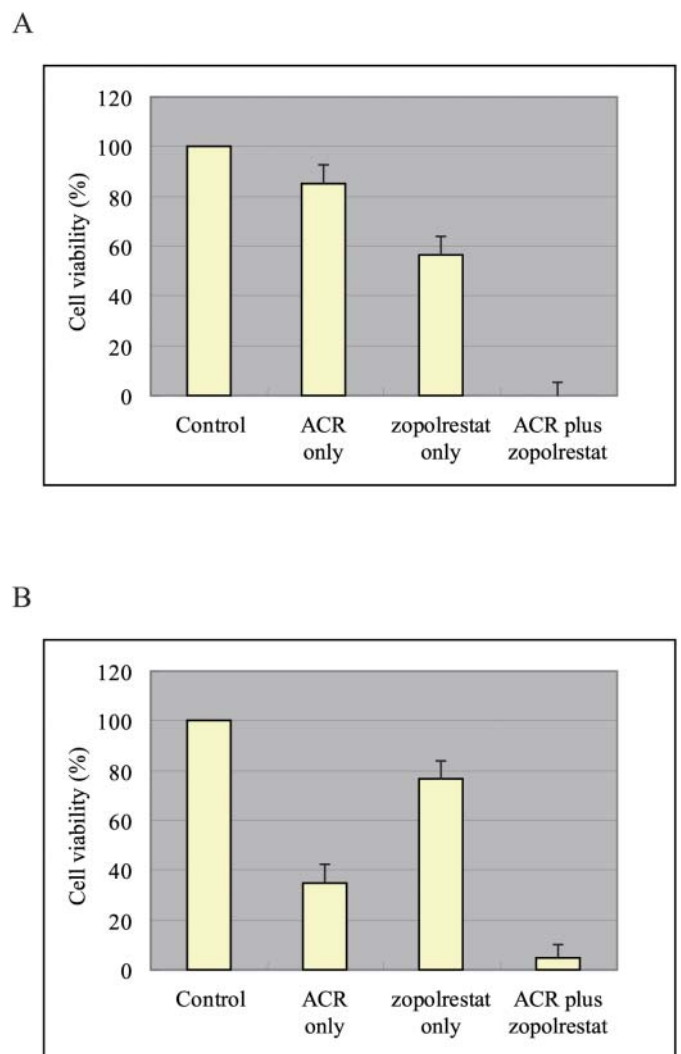


Figure 2 | (A) AKR1B10-positive liver cancer cell viability after 6 days. (B) AKR1B10-negative liver cancer cell viability after 6 days.

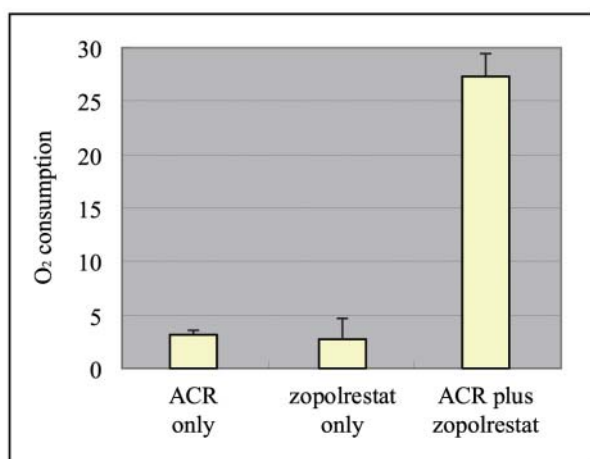
was also assessed 3 days after the administration of ACR alone, zopolrestat alone and ACR plus zopolrestat. Oxygen consumption was significantly greater in AKR1B10-negative liver cancer cells treated with ACR plus zopolrestat than in the cells treated with either ACR alone or zopolrestat alone (Figure 3B; $P < 0.001$, Mann-Whitney U test).

Discussion

HCC has a high recurrence rate (49% in 38 months after curative therapy) and a poor prognosis²⁻³; no effective treatment has been established for recurrent HCC²⁻³. This study of the *in vitro* anticancer drug sensitivity of AKR1B10-positive cells from patients with HCC and ACR resistance (Figures 2A and 3A) suggests that AKR1B10-positive patients with HCC and ACR resistance should be treated with ACR plus zopolrestat rather than with ACR alone to reduce HCC recurrence. Moreover, our experimental results support the hypothesis⁵ that AKR1B10 inhibits the cellular differentiation induced by RA.

AKR1B10 has been reported to act as the major retinaldehyde reductase, thereby decreasing cellular levels of RA⁸⁻¹¹. This action may be the molecular basis underlying the combined effects of ACR plus zopolrestat. However, no studies have reported that AKR1B10 acts on RA or ACR. Therefore, although it is reasonable to postulate that zopolrestat inhibits AKR1B10 in human HCC cells

A



B

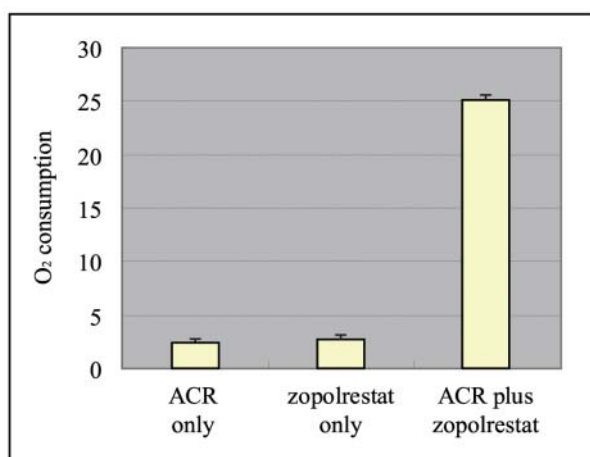


Figure 3 | (A) Oxygen consumption in the AKR1B10-positive liver cancer cells 3 days after the administration of ACR alone, zopolrestat alone and ACR plus zopolrestat. (B) Oxygen consumption in the AKR1B10-negative liver cancer cells 3 days after the administration of ACR alone, zopolrestat alone and ACR plus zopolrestat.

and potentiates the endogenous RA anti-cancer activity, this mechanism cannot be adapted to the combination of ACR plus an AKR1B10 inhibitor unless we can demonstrate that AKR1B10 has ACR reductase activity. Considering these points and our experimental results, we believe that the combined effects of ACR plus zopolrestat can be attributed to the synergism between ACR and 9-cis-RA^{12–13}, in which ACR prevented hyperphosphorylation and the inactivation of RXR α , and thereby restored 9-cis-RA activity. Thus, zopolrestat may prevent endogenous 9-cis-RA depletion, whereas ACR prevents RXR α inactivation. The AFP and albumin values in AKR1B10-positive or negative liver cancer cells improved after treatment with ACR plus zopolrestat, and approached the values that are expected from normal hepatocytes. Moreover, as described by Otto Warburg in 1931, although cancer cells preferentially utilize glycolytic pathways for energy generation while downregulating their aerobic respiratory activity¹⁴, oxygen consumption in AKR1B10-positive or -negative liver cancer cells increased with ACR plus zopolrestat (Figures 3A and 3B). Therefore, the combined effects of ACR plus zopolrestat may work efficiently in the direct

reprogramming and destruction of human HCC cells. In addition, our results suggest that even in patients with HCC and ACR resistance who possess AKR1B10-negative liver cancer cells, ACR plus zopolrestat therapy would be desirable to reduce the rate of recurrence of HCC (Figures 2B and 3B).

This therapeutic method may also be applicable to other human solid tumor cells (i.e., HCC cells, non-small cell lung cancer cells from smokers⁴ or cervical cancer cells¹⁵, etc.) that express AKR1B10 and RXRs. However, in the near future, clinical studies and/or clinical trials will be necessary to investigate the efficacy and safety of ACR plus zopolrestat for human solid tumor cells that express AKR1B10 and RXRs.

In conclusion, these results confirm that liver cancer cells with ACR resistance were directly reprogrammed to approximate normal human hepatocytes, and ACR plus zopolrestat induced apoptosis (Figures 2A, 2B, 3A and 3B). Therefore, a therapeutic method to reprogram and destroy human solid tumor cells with chemicals alone has been developed.

Methods

Chemicals. Acyclic retinoid (all *trans*-3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid: ACR) and zopolrestat were provided by Kowa (Tokyo, Japan).

Patients. In our study, 10 liver cancer samples were obtained with informed consent from patients who underwent a liver transplantation at our institutions (Ref. 6). The 10 patients with HCC before a liver transplantation (Ref. 6) had an age range of 34–60 years (median: 55.7). All of the males ($n=7$) were diagnosed with stage II cancer and were HCV-positive with a Child-Pugh score of A; all of the females ($n=3$) were diagnosed with stage III cancer and were HCV-positive with a Child-Pugh score of B.

A statement identifying the institutional and/or licensing committee experimental approval. The study was approved by the Institutional Review Boards of our institutions (Harvard Medical School, the University of Tokyo, Tokyo Medical and Dental University), and informed written consent was obtained from all of the patients.

The evaluation of AKR1B10 expression. We used a previously described method⁴ to investigate AKR1B10 expression in liver cancer cells from 10 patients with HCC who received a liver transplantation⁶. In this study, an AKR1B10 antibody (monoclonal rabbit anti-human AKR1B10 antibody, clone 1A6; Abnova Corporation) was used. AKR1B10 was evaluated in the human HCC samples according to the number of positively stained tumor cells. If none of the tumor cells demonstrated AKR1B10 immunostaining, the sample was considered to be AKR1B10-negative. The following primer and probes were used: Sequence (5' to 3')

AKR1B10-F: CATATCCAGAGGAATGTGATTGTCA;
AKR1B10-R: AGACCTGAATGTTCTCAACATTGC

In vitro anticancer drug sensitivity testing for the 10 patients with HCC. The liver cancer cells were incubated in a flask coated with collagen gel in a CO₂ incubator at 37°C for 24 h. Only the viable cells adhering to the collagen gel were collected and resuspended in the reconstituted type 1 collagen solution for a final density of 1×10^5 cells/mL. Three drops of the collagen-cell mixture were placed in each well of a 6-well plate in a 60 mm dish, and the plates were allowed to reach 37°C in a CO₂ incubator for 1 h. The final concentration was approximately 3×10^4 cells/collagen gel droplet. Culture medium was added to each well, and the plate was incubated in a CO₂ incubator at 37°C overnight. The anticancer drugs (i.e., 10 μ M ACR alone, 10 μ M zopolrestat alone or 10 μ M ACR plus 10 μ M zopolrestat) were added to the cells for 2 days.

Determination of albumin and alpha fetoprotein (AFP) in the culture media. The albumin protein concentration was determined by an enzyme-linked immunosorbent assay (ELISA) using goat anti-human albumin (Cosmo Bio Co., Tokyo, Japan). The quantitative analysis of AFP secretion was performed with the AFP Amerlex-M RIA detection kit (Ortho Clinical Diagnostics, Issy les Moulineaux, France).

Measurement of oxygen consumption in the liver cancer cells. The oxygen consumption (mean \pm SD, nmol min⁻¹ mg⁻¹ protein) in the AKR1B10-positive or negative liver cancer cells was measured by a Clark-type oxygen microelectrode. Measurements were conducted 3 days after the administration of 10 μ M of ACR alone, 10 μ M of zopolrestat alone, and 10 μ M of ACR plus 10 μ M zopolrestat.

Statistical analyses. All statistical tests were performed with Dr. SPSS II for Windows (SPSS Japan, Inc, Tokyo), and statistical significance was defined as $p < 0.05$.

- Okita, K. *et al.*, A more efficient method to generate integration-free human iPS cells. *Nat. Methods* 8, 409–412 (2011).



2. Muto, Y. *et al.*, Prevention of second primary tumors by an acyclic retinoid, polypropenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. *N Engl J Med* **334**, 1561–1567 (1996).
3. Okita, K. *et al.*, Effect of peretinoin on recurrence of hepatocellular carcinoma (HCC): Results of a phase II/III randomized placebo-controlled trial. *J Clin Oncol* **28**, 15s (Suppl: Abstract 4024) (2010).
4. Fukumoto, S. *et al.* Overexpression of the aldo-keto reductase family protein AKR1B10 is highly correlated with smokers' non-small cell lung carcinomas. *Clin Cancer Res* **11**, 1776–1785 (2005).
5. Penning, T. M. AKR1B10: a new diagnostic marker of non-small cell lung carcinoma in smokers. *Clin Cancer Res* **11**, 1687–1690 (2005).
6. Moriguchi, H., Chung, R. T., Mihara, M. & Sato, C. The generation of human induced pluripotent stem (iPS) cells from liver progenitor cells by only small molecules and the risk for malignant transformations of the cells. *ACSC* **2**, 5–9 (2010). (<http://www.jstage.jst.go.jp/browse/acsc/>)
7. Balendiran, G. K., Martin, H. J., El-Hawari, Y. & Maser, E. Cancer biomarker AKR1B10 and carbonyl metabolism. *Chem Biol Interact* **178**, 134–137 (2009).
8. Crosas, B., Cederlund, E., Torres, D., Jornvall, H., Farres, J., Pares, X. A vertebrate aldo-keto reductase active with retinoids and ethanol. *J Biol Chem* **276**, 19132–19140 (2001).
9. Gallego, O. *et al.* Comparative functional analysis of human medium-chain dehydrogenases, short-chain dehydrogenases/reductases and aldo-keto reductases with retinoids. *Biochem J* **399**, 101–109 (2006).
10. Gallego, O. *et al.* Structural basis for the high all-trans-retinaldehyde reductase activity of the tumor marker AKR1B10. *Proc Natl Acad Sci U S A* **104**, 20764–20769 (2007).
11. Ruiz, F. X. *et al.* Aldo-keto reductases from the AKR1B subfamily: retinoid specificity and control of cellular retinoic acid levels. *Chem Biol Interact* **178**, 171–177 (2009).
12. Matsushima-Nishiwaki, R., Okuno, M., Takano, Y., Kojima, S., Friedman, S. L., Moriaki, H. Molecular mechanism for growth suppression of human hepatocellular carcinoma cells by acyclic retinoid. *Carcinogenesis* **24**, 1353–1359 (2003).
13. Tatsukawa, H. *et al.* Dual induction of caspase 3- and transglutaminase-dependent apoptosis by acyclic retinoid in hepatocellular carcinoma cells. *Mol Cancer* **10**, 4 (2011).
14. Warburg, O. On respiratory impairment in cancer cells. *Science* **124**, 269–270 (1956).
15. Yoshitake, H. *et al.* Aldo-keto reductase family 1, member B10 in uterine carcinomas: a potential risk factor of recurrence after surgical therapy in cervical cancer. *Int J Gynecol Cancer* **17**, 1300–1306 (2007).

Acknowledgements

We are grateful to Professor Klas G. Wiman (Department of Oncology-Pathology, Cancer Center Karolinska, Karolinska Institutet, Stockholm), Dr. Ramond T. Chung (Massachusetts General Hospital and Harvard Medical School, USA), Ms. Satoko Iioka, Ms. Midori Okabe and Mr. Akira Fujimoto.

Author contributions

H.M.: Conception and design, provision of study material, collection and/or assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript; C.S.: provision of study material, collection and/or assembly of data, data analysis and interpretation, manuscript assessment, and final approval of manuscript; Y.Z. and M.M.: data analysis and interpretation, final approval of manuscript.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

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How to cite this article: Moriguchi, H., Zhang, Y., Mihara, M. & Sato, C. A therapeutic method for the direct reprogramming of human liver cancer cells with only chemicals. *Sci. Rep.* **2**, 280; DOI:10.1038/srep00280 (2012).